GENETIC ARCHITECTURE OF ADAPTATION TO BIOTIC INVASIONS IN SOAPBERRY BUGS

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Saskatoon

By
Yue Yu

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ABSTRACT

On the Florida peninsula, the soapberry bug (*Jadera haematoloma*) has been able to colonize the golden rain tree, *Koelreuteria elegans*, since the introduction of this invasive tree only a few decades ago. The populations feeding on the new host have been rapidly differentiating from the native populations. Possibly the most dramatic differentiation is that of the beak (mouthpart) length. Derived populations showed shorter beaks more appropriate for feeding on the flattened pods of the new host. Previous studies have shown that the divergence of the beak length has a genetic basis and involves non-additive genetic effects. However, to date, the soapberry bug divergence has not been studied at the molecular level. In the current study, I have generated a three-generation pedigree from crossing the long-beaked and short-beaked ecomorphs to construct a *de novo* linkage map and to locate putative QTL controlling beak length and body size in *J. haematoloma*. Using the AFLP technique and a two-way pseudo-testcross design I produced two parental maps. The maternal map covered six linkage groups and the paternal map covered five; the expected number of chromosomes was recovered and the putative X chromosome was identified. For beak length, QTL analyses revealed one significant QTL. Three QTL were found for body size. Interestingly, the most significant body size QTL co-localized with the beak length QTL, suggesting linkage disequilibrium or pleiotropic effects of related traits. Through single marker regression analysis, nine single markers that could not be placed on the map were also found to be associated with either trait. However, I found no evidence for epistasis. Overall, my findings support an oligogenic model of genetic control on beak length and body size, and the underlying genetic architectures were complex. This study is the first to look at the molecular basis underlying adaptive traits in the soapberry bug, and contributes to understanding of the genetic changes involved in early stages of ecological divergence.

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LIST OF ABBREVIATIONS AND SYMBOLS

AFLP——————Amplified Fragment Length Polymorphism

ANCOVA———Analysis of Covariance

DNA——————Deoxyribonucleic Acid

LG——————Linkage Group

PCR——————Polymerase Chain Reaction

QTL——————Quantitative Trait Locus

SNP——————Single Nucleotide Polymorphism

℃——————degree Celsius

bp——————base pair

ng——————nanogram

cM——————centimorgan

Gb——————giga base pair

µL——————microlitre

µM——————micromolar

min——————minute

mm——————millimeter

s——————second

CHAPTER 1 - GENERAL INTRODUCTION

1.1 Ecological Divergence

Species diversity is one of the central interests in evolutionary biology. Most studies in the past have looked at fixed differences between closely related species (e.g., Roff & Mousseau 1999; Panhuis *et al.* 2001; Andrés *et al.* 2006). However, it poses a challenge disentangling differences leading up to reproductive isolation from differences that have accumulated thereafter. An emerging idea is that instead of taking a retrospective approach to study speciation, intraspecific diverging lines under ecologically-based divergent selection (ecomorphs) could be used as model systems to provide insight into early stages of ecological speciation (Schluter 2001; Rundle & Nosil 2005; Via & West 2008; Feder *et al.* 2012). Naturalized plants often attract one or more resident herbivores (Frenzel & Brandl 2003; Graves & Shapiro 2003; Carroll & Loye 2012; Pearse & Altermatt 2013), and because these events can be monitored close to the time of introduction, they have proven to be key to unraveling the genetic origins of new lineage formation (Bush 1969; Via 1991; Carroll *et al.* 1997, 2005; Feder *et al.* 2003; Schwarz *et al.* 2005; Gompert *et al.* 2006).

At the genome level, several models of adaptive divergence have been proposed, ranging from widespread differentiation across the genome by means of linkage disequilibrium with the essential genes responsible for ecologically important traits, to differentiation restricted to a few "islands" surrounding those genes due to limited recombination. Host-races of phytophagous insects under divergent selection in the presence of several potential plant hosts have been popular systems to construct such predictive models (Berlocher & Feder 2002; Martel *et al.* 2003; Egan *et al.* 2008; Nosil *et al.* 2008; Peccoud *et al.* 2009; Singer & McBride 2010).

1.2 The Study System: *Jadera haematoloma*

The soapberry bug comprises a heteropteran subfamily (Serinethinae) that specializes on

plants from the family Sapindaceae. Species of the soapberry bug have a world-wide distribution in the neotropic ecozone. In particular, the model species of the current study, *Jadera haematoloma*, is found from Colombia in South America up to the southern half of the United States of America. Like other members of Hemiptera, the soapberry bug's mouthparts are of the sucking type, in which the labium encloses the mandibles and maxillae and together they form a proboscis for piercing and sucking the sapind seeds. The soapberry bug proboscis is also referred to as the beak.

The soapberry bug is undergoing rapid divergence in the face of ecological changes brought by anthropogenic activities (Carroll 2007, 2008). In Florida, U.S.A., the native host plant to *Jadera haematoloma* is the balloon vine (*Cardiospermum corindum*). The golden rain tree (*Koelreuteria elegans*) was introduced to Florida as an ornamental tree several decades ago. In the few decades the bug has diverged into two ecomorphs associated with each host, and differentiated on traits such as life history, host preference, and morphology (Carroll & Boyd 1992; Carroll *et al.* 1997, 2001; Carroll, Marler, *et al.* 2003). Carroll & Boyd (1992) found that the difference in beak length between the ecomorphs reflected the difference in fruit capsule size between the host plants. A longer beak is needed for a larger fruit capsule, such as that of the balloon vine with an inflated shape, due to the longer distance between the fruit wall and the seeds in the center. Similar but independent cases (involving other soapberry bug species) could be found elsewhere in North America and Australia (Carroll & Boyd 1992; Carroll *et al.* 2005).

Laboratory experiments found that the differences in beak length between ecomorphs were largely due to genetic factors (Carroll *et al.* 1997; Dingle *et al.* 2009). Further cross-breeding experiments suggested significant effects of non-additive genetic control, and that each differentiated trait had a different genetic architecture (Carroll *et al.* 2001; Carroll, Dingle, *et al.* 2003). However, there are no existing studies at the molecular level for characterizing such genetic architectures.

1.3 Research Objectives

Being an organism under rapid ecological divergence facing human-induced biotic invasions, the soapberry bug presents an interesting case for studying the genetic basis to adaptive divergence. The ultimate goal is to characterize the genetic architecture underlying adaptive divergence in *Jadera haematoloma* in Florida. To do so, I will focus on the beak length, an ecologically important trait, and identify genomic regions controlling beak length by achieving two objectives as described below.

1.3.1 Objective 1: To generate a genome-wide linkage map using AFLP markers

First a cross between the ecomorphs is performed so that recombination rates between genetic markers (genetic distances) may be estimated. Those quantitative trait loci (QTL) that segregate in the mapping family may potentially be identified in later analyses.

To date, the soapberry bug has not been studied at the molecular level, and does not have any closely related species that has a reference genome. As such, a linkage map has to be constructed *de novo* based on the mapping family.

For genotyping, Amplified Fragment Length Polymorphism (AFLP), a fingerprinting technique that characterizes genomic differences without needing sequencing, will be used to develop restriction-site-associated markers across the genome for constructing the linkage map.

1.3.2 Objective 2: To map QTL underlying the beak length

By studying the association between genotype and phenotype while taking into account the genetic distances between markers, locations of putative QTL may be estimated.

CHAPTER 2 - LINKAGE AND QTL MAPPING IN *JADERA HAEMATOLOMA*[1]

2.1 Introduction

Adaptive evolution plays a key role in biotic invasions. When a species is introduced to a new habitat it is likely to experience new selective pressures, and populations of invaders frequently experience rapid evolutionary changes (e.g. Mooney & Cleland 2001; Lee 2002; Lambrinos 2004; Suarez & Tsutsui 2008; Shine 2012). Simultaneously, invaders also act as selective agents, often driving evolutionary change in the exposed native populations (reviewed in Strauss *et al.* 2006). Both evolvability (*i.e.* the ability of the genetic system to produce and maintain potentially adaptive genetic variants [Hansen 2006]) and the response of invaders and native species to selection critically depend on the genetic architecture of ecologically relevant traits (see Colautti *et al.* 2010, 2012).

One measure of genetic architecture is the G matrix (Lynch & Walsh 1998), which is composed of genetic variances and covariance among traits sharing developmental and genetic processes. The G matrix can rapidly evolve in natural populations (Doroszuk *et al.* 2008). However, to our knowledge, the only study comparing G matrices between native and invasive populations (Calsbeek *et al.* 2011) suggested that, at least in this case, the molecular-genetic underpinnings of the matrix elements are similar between invasive and native populations. A second measure of genetic architecture is the estimate of the relative effects of additive and non-additive (dominant, epistatic, and pleiotropic) genetic variance on individual traits. Though it is generally assumed that the response to selection relies only on the existence of additive genetic variance, gene interactions may play a central role in contemporary evolution because directional epistasis makes gene effects become evolvable and enables rapid changes in additive

[1] This chapter has been submitted to Genetics for peer review (Yu & Andrés, 2013; MS ID 156489). As first author, I generated the mapping family, carried out the genotyping and statistical analyses, and wrote the draft of the manuscript.

effects and evolvability (Carter *et al.* 2005; Hansen 2006). In invasive species, non-additive genetic variance seems to play a key role during the colonization of new habitats (see Lee 2002). Similarly, research on native phytophagous insects shifting onto introduced hosts has highlighted the role of epistasis and other non-additive genetic effects in the rapid colonization of the invasive hosts (Carroll *et al.* 2001; Carroll, Dingle, *et al.* 2003; Carroll 2007). The third measure of genetic architecture is the dissection of trait variation into its genomic components facilitated by advances in molecular genetics. Quantitative trait locus (QTL) mapping can reveal the number and type of genomic regions, and potentially genes, affecting quantitative variation as well as the number of possible gene interactions. To date, only a few studies have used QTL to look at the genetic basis of "invasiveness" (Linde *et al.* 2001; Weinig *et al.* 2007) and to our knowledge QTL mapping has not yet been used to look at evolutionary responses of native species to invasions.

Host shifts of phytophagous insects represent the best body of evidence for the rapid evolution of native species in response to the introduction of novel species (Strauss *et al.* 2006). Here we focus on an anthropogenic host-shift in the soapberry bug, *Jadera haematoloma*, and use a QTL approach to study the genetic architecture of beak length, a complex, heritable trait that is closely associated with the species' ability to colonize new hosts.

Soapberry bugs comprise a subfamily of three widespread genera of seed predator bugs that have become a textbook example of evolution in action (e.g. Moore & Moore 2006; Futuyma 2013; Freeman & Herron 2013). These insects exploit a broad variety of host plants from the family Sapindaceae (Carroll 2007). In North America and Australia different species of soapberry bug show ongoing rapid evolution of their beaks to better match the seed defense structures of newly introduced hosts (Carroll & Boyd 1992; Carroll *et al.* 1997; Dingle *et al.* 2009). On the Florida peninsula, populations of the Neotropical soapberry bug *J. haematoloma* feed on the seeds of both the native balloon vine (*Cardiospermum corindum*) and the invasive Taiwanese golden rain tree (*Koelreuteria elegans*), introduced into urban areas about 70 years

ago. These two host species differ in fruit size, phenology and seed chemical defenses (Seigler & Kawahara 1976; Carroll & Boyd 1992; Carroll *et al.* 1998; Carroll, Dingle, *et al.* 2003). Driven by selection as the result of these differences the populations feeding on the newly colonized tree (*K. elegans*) have evolved into the "derived" ecomorph. Several morphological, physiological and behavioral differences exist between the ancestral and derived *J. haematoloma* ecomorphs. Possibly the most striking one is the reduction of beak length appropriate to exploit the flatter fruits of the invasive tree (Carroll *et al.* 1998, 2001; Carroll, Dingle, *et al.* 2003; Dingle *et al.* 2009). Controlled crosses, common garden and artificial selection experiments have shown that beak size differences are heritable, that beak length is controlled by multiple genes, and that epistatic interactions are likely to play a key role in the evolution of shorter beaks (Carroll *et al.* 2001; Carroll 2007; Dingle *et al.* 2009). This study represents the first attempt to identify the location, number, and effect of the genomic regions associated with beak length, a trait that plays a central role in the trophic diversification of heteropterans.

2.2 Materials and Methods

2.2.1 Mapping population

For this study we collected soapberry bugs in two allopatric populations in Florida (Figure 1). In Key Largo (25° 6' 11.40", -80° 26' 2.88") we collected individuals with long beaks feeding on the native balloon vine (*Cardiospermum corindum*). We collected short beak individuals feeding on the introduced golden rain tree (*Koelreuteria elegans*) in a locality near Orange City (northern Florida; 28° 57' 8.52", -81° 18' 19.50"). The Euclidean distance between these two populations is 437 km. Therefore, although adult bugs are relatively good flyers, gene flow between these two populations is likely to be negligible.

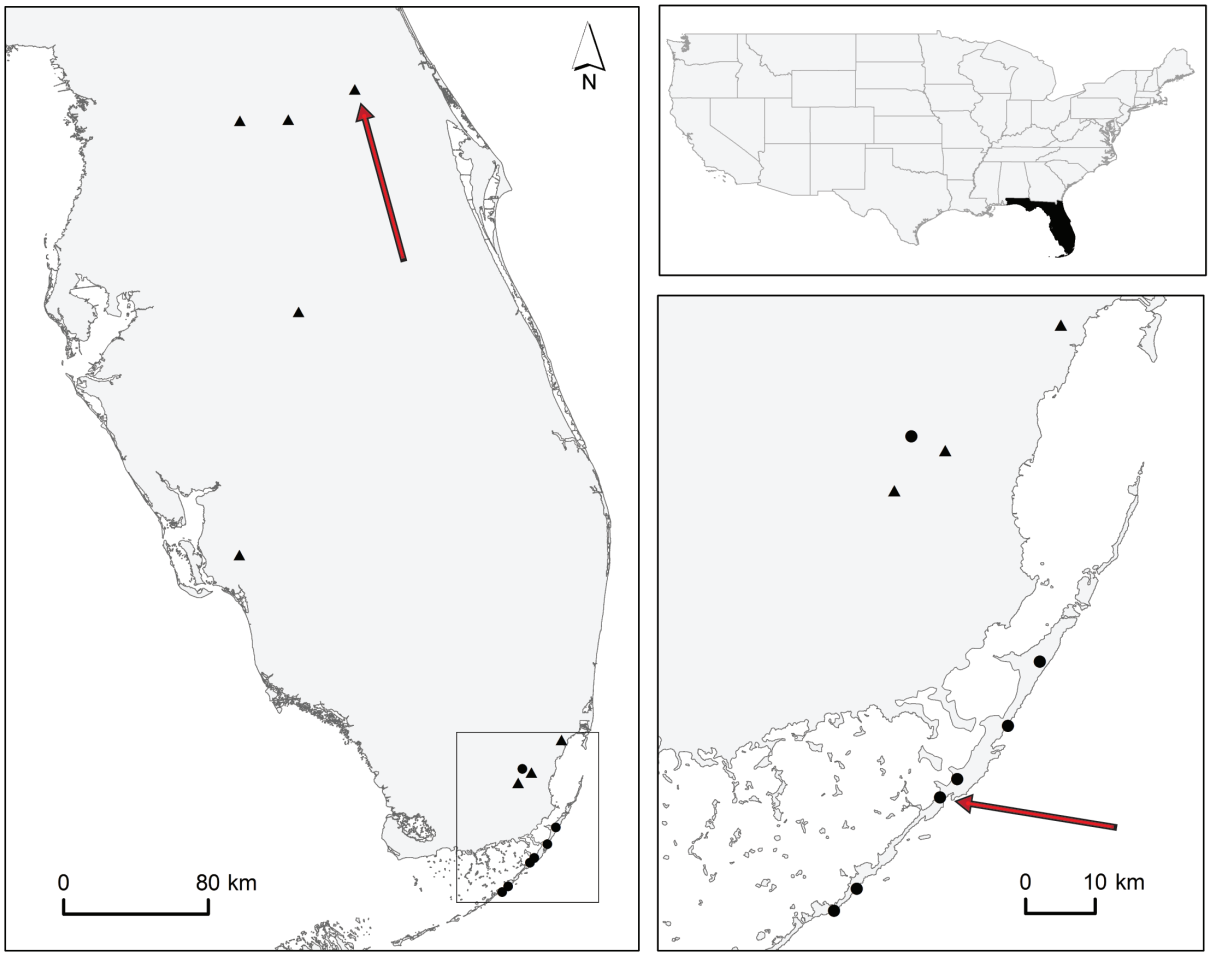


Figure 1: *Jadera haematoloma* populations and sampling sites. Upper right: map of the United States of America with Florida hightlighted. Left: Florida. Lower right: close-up of the Florida Keys and part of the tip of the peninsula. Black circles represent known populations of the ancestral long-beaked ecomorph feeding on the native balloon vine (*C. corindum*). Black triangles represent known populations of the derived short-beaked ecomorph feeding on the introduced golden rain tree (*K. elegans*). Arrows indicate the sampling sites where parents for the experimental cross were sampled.

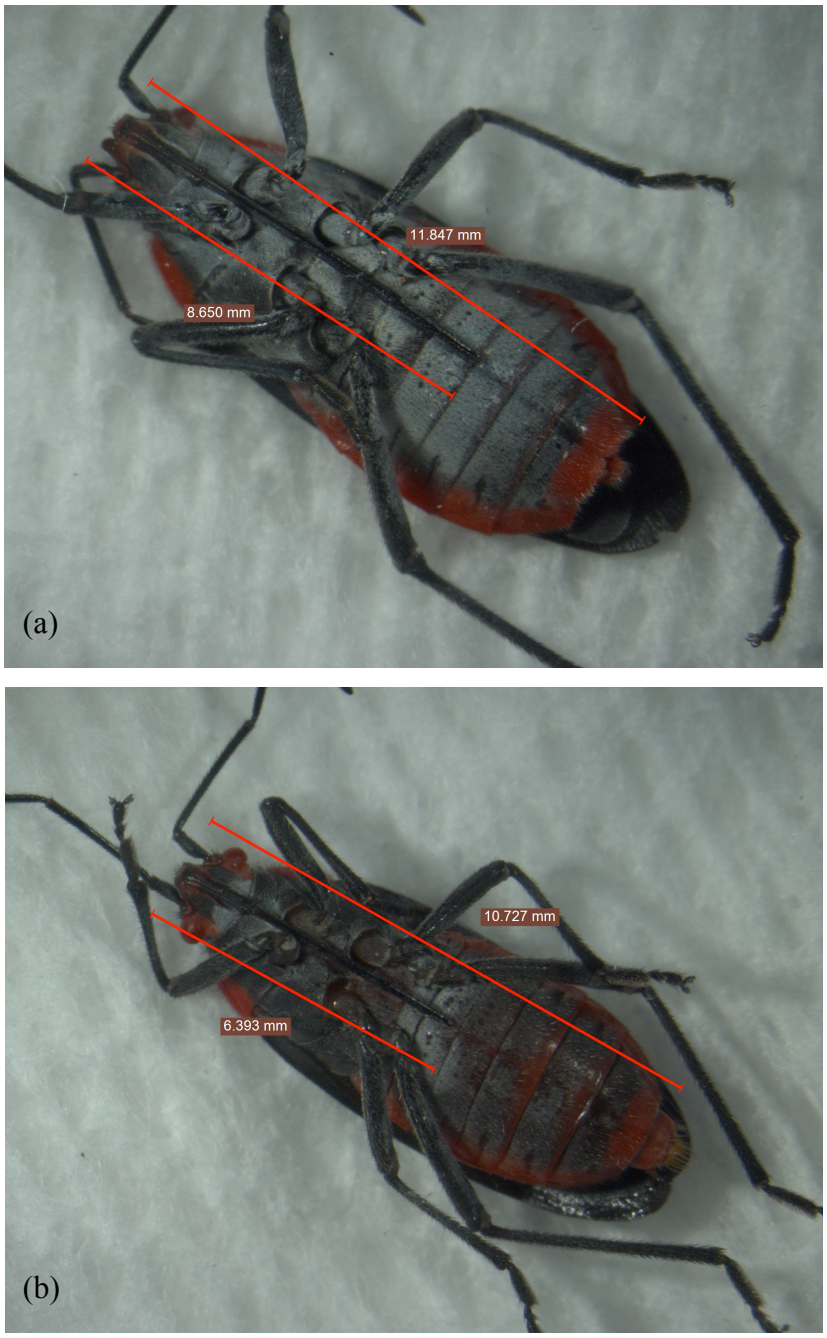


Figure 2: Examples of measuring the beak and body lengths of *Jadera haematoloma* using a digital Leica MZ16A stereomicroscope. (a) Female. (b) Male.

Field collected individuals were maintained in the laboratory on commercially available seeds of *Koelreuteria paniculata* using controlled light and temperature conditions similar to that of the field collecting sites (13.5 hours of daylight at 29°C, 10.5 hours of night time at 20°C, fluorescent tubes). Populations were bred separately in the laboratory for the first generation. A cross between a first-generation long beak virgin female and a first-generation short beak male were crossed to produce F1. Then an F2 mapping population was established from a single pair of F1 full siblings. We sexed each individual and measured the body and beak (labial) lengths using a digitally calibrated Leica MZ16A stereomicroscope (Figure 2). All individuals were then stored at -20°C in 100% ethanol for subsequent genetic analyses.

2.2.2 Fluorescent AFLP methods

We developed AFLP markers following Vos *et al.* (1995) with fluorescently labeled primers (Hartl & Seefelder 1998; Huang & Sun 1999; Ashikawa *et al.* 1999; Trybush *et al.* 2006). For each individual bug we extracted genomic DNA from the thorax using MasterPure™ DNA Purification Kit (Epicentre) following manufacturer's instructions. Approximately 100 ng of DNA of each sample was digested with 5 units *Eco*RI and 5 units *Mse*I (New England BioLabs) for 30 min at 37°C in x1 NEB buffer 4 and BSA in a total reaction volume of 30 µL. Next, to ligate the resulting fragments to the adapters, we added 0.5 µM *Eco*RI adapter, 5 µM *Mse*I adapter, and 60 cohesive end units of T4 DNA ligase (New England Biolabs) in a total volume of 10 µL to the 30 µL of digestion reaction mixture. After incubation (30°C for 90 min), we diluted the samples 10 times with ddH_2O, and used 2.5 µL of each sample as a template to conduct the pre-selective PCRs in a total reaction volume of 10 µL (1x PCR buffer, 0.5µM each of either *Eco*RI-C or *Eco*RI-G combined with each of *Mse*I-C, *Mse*I-G, or *Mse*I-TC primers, 0.2 mM dNTPs, and 0.5 units Qiagen Top-*Taq*® DNA polymerase; 72°C for 150 s followed by 94°C for 3 min, then 22 cycles of [94°C for 30 s, 56°C for 1 min, and 72°C for 1 min], and finally 72°C for 10 min). We diluted these pre-amplified products 1:20 and used them as the template for

selective PCR amplifications in 10 μL (1x PCR buffer, 0.5μM of an *Eco*RI selective primer and an *Mse*I selective primer, 0.2 mM dNTPs, and 0.5 units Qiagen Top-*Taq*® DNA polymerase) using a touchdown protocol (95°C for 3 min, 13 cycles of [94°C for 30 s, 65°C for 30 s with -0.7°C/cycle, and 72°C for 1 min], 12 cycles of [94°C for 30s, 56°C for 30 s, and 72°C for 1 min], and finally 72°C for 10 min). After pre-screening, we selected sixteen combinations of *Eco*RI $^{6\text{-FAM}}$-*Mse*I primers that generated clear fluorescent peaks (CTC-CAAG, CTC-CCTA, CTC-CGAC, CTC-CTGC, GAC-CAAG, GAC-CCTA, GAC-CGAC, GAC-CTGC, CAT-GGAT, CAT-GATC, CAT-GCCA, CAT-GTTC, CCA-TCCA, CCA-TCGC, CCA-TCAT, CCA-TCTG). These primers are similar to those designed for other insect species but *Mse*I primers contained four selective nucleotides to help reduce fragment size homoplasy. To assess the reproducibility of our method the above protocol (including DNA extractions) was repeated on both parents and grandparents. Only clearly repeatable peaks were used in the construction of the map.

To prepare DNA fragments for separation by capillary electrophoresis, a sample loading solution was prepared by mixing 0.1 μL of 600-LIZ size standard® (Applied Biosystems) with 8.9 μL of Hi-Di Formamide, and 1 μL of 1:30 dilution of selective PCR amplification product. Samples were analyzed in ABI 3130*xl* Genetic Analyzer (Applied Biosystems). The presence or absence of fragments was initially scored automatically using GeneMapper v4.1 (Applied Biosystems) with a minimum relative fluorescence unit (RFU) of 30; other parameters were left at default. To further reduce size homoplasy we only scored fragments within the 90–550 bp size range (Caballero *et al.* 2008; Paris *et al.* 2010). Bin and peak calls were then confirmed upon manual inspection.

2.2.3 Genetic linkage analysis and map construction

Polymorphic, repeatable AFLP markers were classified into different segregation classes depending on the allele patterns of the parents. In total, we defined three marker classes using the CP (outbreeding species full-sibling family) population type implemented in JoinMap® 4.0 (Van

Ooijen 2006): (1) markers that segregate only in the mother (*lm* × *ll*), (2) markers that segregate only in the father (*nn* × *np*), and (3) markers that segregate in both parents (*hk* × *hk*). The expected segregation ratios were 1:1 for the first two classes and 3:1 for the last one. To evaluate any discrepancy from the expected segregation ratios we used the χ^2 goodness-of-fit method as implemented in JoinMap® 4.0. Markers showing segregation distortion at the significance level of $P = 0.05$ were excluded from further analyses. Linkage groups were determined using a LOD threshold of 4.0. Map construction was performed using the Kosambi mapping function and the regression mapping algorithm. Two independent (maternal and paternal) maps were generated using *lm* × *ll* and *nn* × *np* markers, respectively, employing a two-way pseudo-testcross strategy (Grattapaglia & Sederoff 1994). The positions of these markers were taken to be fixed orders to further populate the parental maps with *hk* × *hk* markers segregating in both parents. The *hk* × *hk* markers were then used to compare maternal and paternal linkage groups. To test if the AFLP markers were randomly distributed within linkage groups we used the χ^2 goodness-of-fit method proposed by Voort *et al.* (1997).

2.2.4 QTL analysis

For our QTL analyses we employed the BCF2 module of GridQTL (Seaton *et al.* 2006) available online at *http://www.gridqtl.org.uk*. The statistical approach of this module adopts the methods of Haley *et al.* (1994). It is suitable for crosses between outbred lines and assumes that the alternative alleles at major QTLs affecting the traits of interest are fixed (e.g. lineages with different selection histories). QTL analyses using the TREE module, which does not assume fixed QTL, yielded similar results (data not shown). Significance thresholds were obtained from permutation tests (n = 10000) as described in Churchill & Doerge (1994). We considered a QTL significant if it was detected at either $P < 0.01$ at the chromosome-wide level or $P < 0.05$ at the experiment-wide level. We considered a QTL suggestive if it was only detected at $P < 0.05$ at the chromosome-wide level.

We used a forward and backward selection interval mapping approach for QTL analysis (Guo *et al.* 2008; Leach *et al.* 2012): First, a one-QTL model including the additive and dominant effects of a QTL was fitted at each 1 cM by least square methods for beak and body lengths. If one or more significant or suggestive QTL were detected, the one showing the highest *F*-value was considered to be the first QTL. Second, by using the first QTL as genetic background effects, we searched for QTL of lesser effects in the other linkage groups. In addition, a two-QTL model was fitted to detect any other potential QTL on the same linkage group. Among the significant or suggestive QTL detected at this step, the one with the highest *F*-value was considered as the second QTL. Next, in the backward selection step, we used this new QTL as genetic background effects to re-estimate the position and effects of the first one. Adjusted parameters of the first QTL were used as genetic background effects and the second QTL was again re-assessed. The forward and backward steps were iterated until the parameters for the two identified QTL remained constant. Third, the parameters of the two QTL were used to detect a new QTL. The previous steps were repeated until no new significant or suggestive QTL were found when using all previously detected QTL as genetic background. Finally, we estimated the phenotypic variance explained by each QTL according to the equation of Wang *et al.* (2012).

Additionally, we also conducted single marker regression analysis on markers that were excluded due to segregation distortion, and markers that failed to be grouped with the current linkage groups at an LOD threshold of 4.0 (unlinked). For each marker, phenotypic values (beak or body length) were separated into two groups based on the genotypes (presence or absence of the AFLP fluorescence peak), and Analysis of Covariance (ANCOVA), taking sex as a covariate, was used to find significant difference at $P < 0.05$. For those markers found to be significant, we estimated the percentage of phenotypic variance explained by each marker using the equation:

$$V_{EXPLAINED} = SS_{marker} / (SS_{total} - SS_{sex}) \times 100\%$$

Where SS_{marker} is the sum of squares absorbed by the marker after adjusting for the covariate sex

in the full model, SS_{total} is the corrected total sum of squares in the null model, and SS_{sex} is the sum of squares absorbed by sex alone in the reduced model.

To detect any potential QTL × sex interactions we included a sex interaction term into the model and we estimated both additive and dominance effects of the QTL in each sex using GridQTL. We considered that significant sex differences in the estimates of the QTL effects are indicative of QTL × sex interactions. Finally, to detect QTL with epistatic effects we first imported the genotypic probabilities for each 1 cM calculated by GridQTL into R/qtl using outbred.qtl (R package; Nelson *et al.* 2011). Then we examined genome-wide evidence for epistasis using the *scantwo* function of R/qtl with the Haley-Knott regression method. LOD significance thresholds were determined by permutation tests (n = 500).

2.3 Results and Discussion

2.3.1 Linkage map and chromosomal behavior

To generate a linkage map we produced 81 F2 individuals (48 females and 33 males) from a single F1 cross between two parental diverging lineages of *J. haematoloma* associated with two different host plants. This species is sexually dimorphic (Carroll & Boyd 1992). Accordingly, the resulting female offspring were on average bigger (mean ± sd: 11.75 mm ± 0.64 mm) than the male offspring (9.99 mm ± 0.36 mm), and female beaks (8.09 mm ± 0.48 mm) were on average longer than those of the males (5.93 mm ± 0.23 mm; Figure 3). As expected, the observed distribution of beak sizes in the experimental cross is intermediate between those observed in natural populations of the parental lineages (see Carroll & Boyd 1992).

Cytogenetically, the soapberry bug (*J. haematoloma*) is characterized by an XX/X0 (female/male) sex determination system, five pairs of autosomal chromosomes, and one pair of m chromosomes ($2n_{female}$=10+2m+XX, $2n_{male}$=10+2m+X0; Bressa *et al.* 2001). The m chromosomes are small, achiasmatic, and behave as univalents during early meiotic stages (Bressa *et al.* 2001, 2005) and, *a priori*, we did not anticipate covering it in our linkage map.

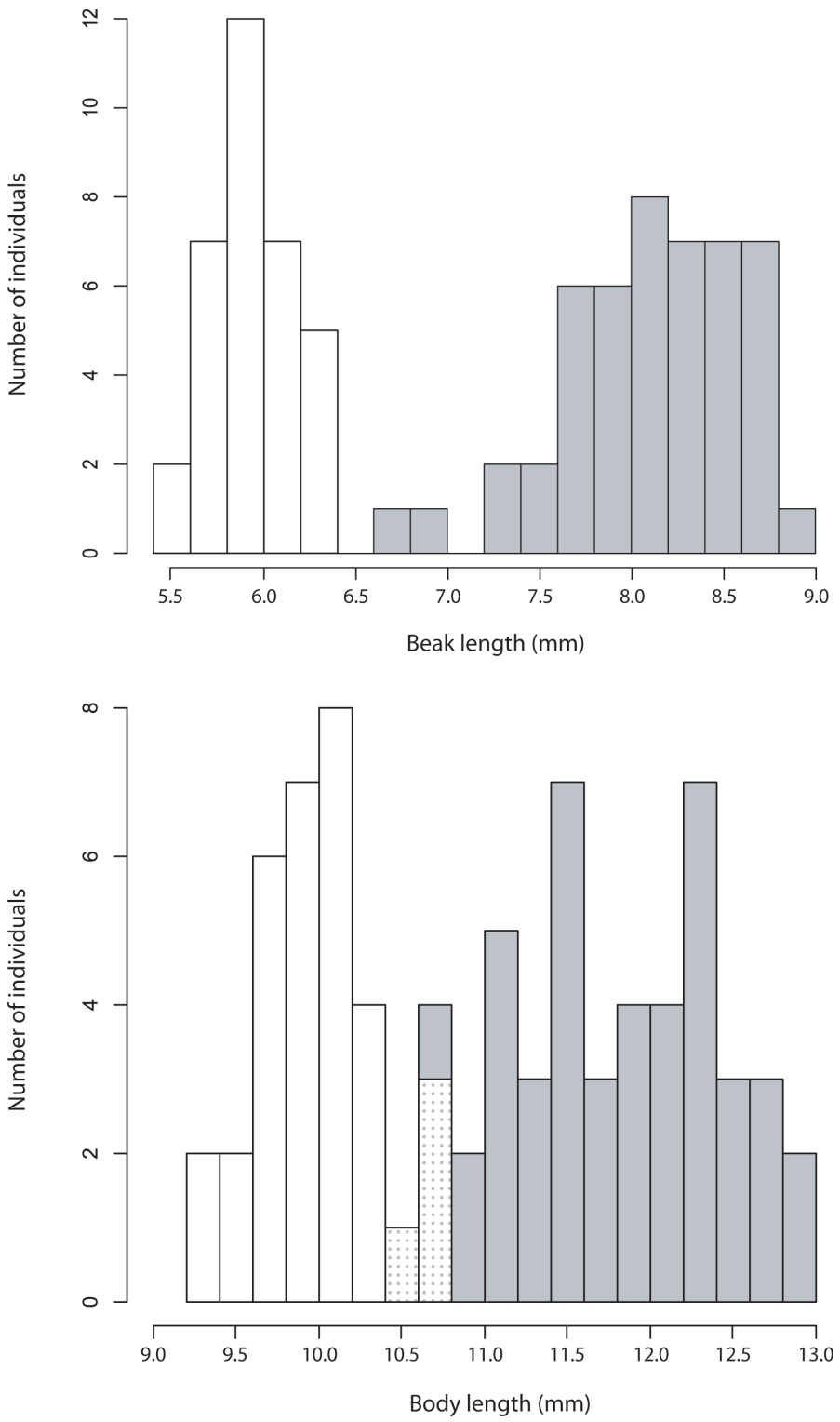
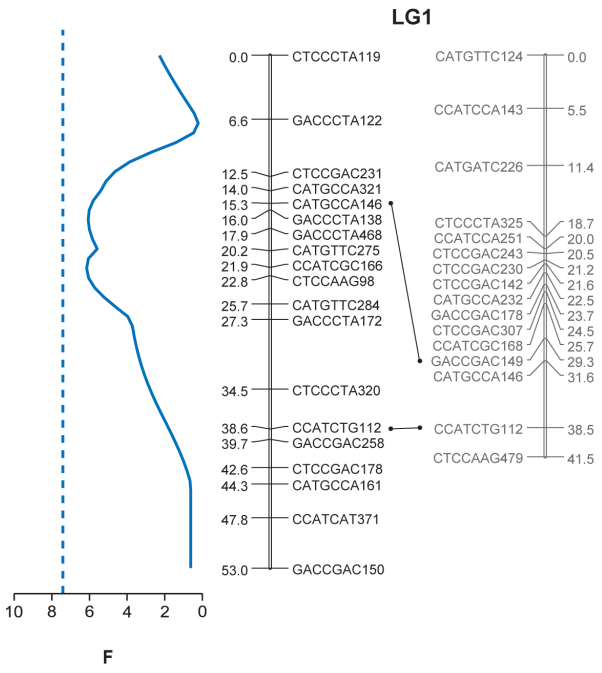
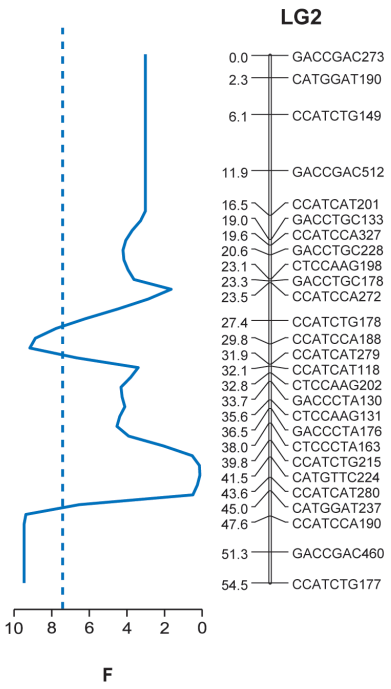
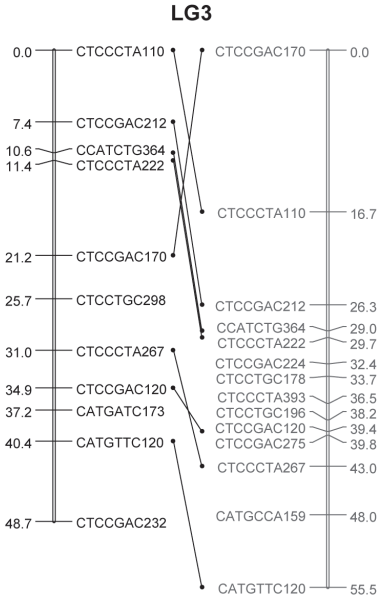
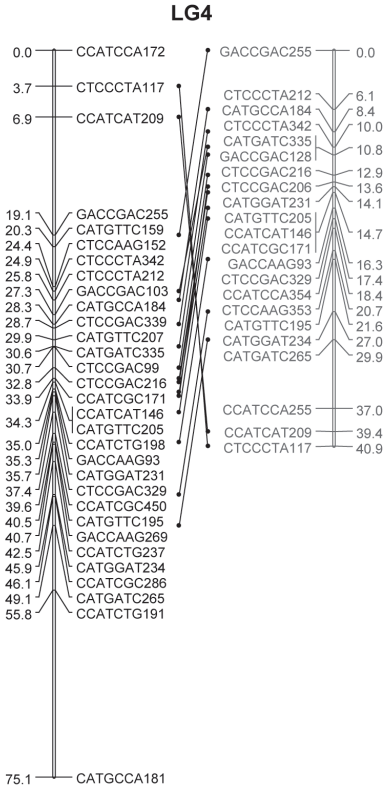


Figure 3: Histograms showing beak length and body length distributions in the mapping family. Grey bars indicate females, white bars indicate males. The dotted areas indicate the overlap between the two.


LG1
0.0 CTCCCTA119
6.6 GACCCTA122
12.5 CTCCGAC231
14.0 CATGCCA321
15.3 CATGCCA146
16.0 GACCCTA138
17.9 GACCCTA468
20.2 CATGTTC275
21.9 CCATCGC166
22.8 CTCCAAG98
25.7 CATGTTC284
27.3 GACCCTA172
34.5 CTCCCTA320
38.6 CCATCTG112
39.7 GACCGAC258
42.6 CTCCGAC178
44.3 CATGCCA161
47.8 CCATCAT371
53.0 GACCGAC150
CATGTTC124 0.0
CCATCCA143 5.5
CATGATC226 11.4
CTCCCTA325 18.7
CCATCCA251 20.0
CTCCGAC243 20.5
CTCCGAC230 21.2
CTCCGAC142 21.6
CATGCCA232 22.5
GACCGAC178 23.7
CTCCGAC307 24.5
CCATCGC168 25.7
GACCGAC149 29.3
CATGCCA146 31.6
CCATCTG112 38.5
CTCCAAG479 41.5
10 8 6 4 2 0
F


LG2
0.0 GACCGAC273
2.3 CATGGAT190
6.1 CCATCTG149
11.9 GACCGAC512
16.5 CCATCAT201
19.0 GACCTGC133
19.6 CCATCCA327
20.6 GACCTGC228
23.1 CTCCAAG198
23.3 GACCTGC178
23.5 CCATCCA272
27.4 CCATCTG178
29.8 CCATCCA188
31.9 CCATCAT279
32.1 CCATCAT118
32.8 CTCCAAG202
33.7 GACCCTA130
35.6 CTCCAAG131
36.5 GACCCTA176
38.0 CTCCCTA163
39.8 CCATCTG215
41.5 CATGTTC224
43.6 CCATCAT280
45.0 CATGGAT237
47.6 CCATCCA190
51.3 GACCGAC460
54.5 CCATCTG177
10 8 6 4 2 0
F


LG3
0.0 CTCCCTA110
7.4 CTCCGAC212
10.6 CCATCTG364
11.4 CTCCCTA222
21.2 CTCCGAC170
25.7 CTCCTGC298
31.0 CTCCCTA267
34.9 CTCCGAC120
37.2 CATGATC173
40.4 CATGTTC120
48.7 CTCCGAC232
CTCCGAC170 0.0
CTCCCTA110 16.7
CTCCGAC212 26.3
CCATCTG364 29.0
CTCCCTA222 29.7
CTCCGAC224 32.4
CTCCTGC178 33.7
CTCCCTA393 36.5
CTCCTGC196 38.2
CTCCGAC120 39.4
CTCCGAC275 39.8
CTCCCTA267 43.0
CATGCCA159 48.0
CATGTTC120 55.5


LG4
0.0 CCATCCA172
3.7 CTCCCTA117
6.9 CCATCAT209
19.1 GACCGAC255
20.3 CATGTTC159
24.4 CTCCAAG152
24.9 CTCCCTA342
25.8 CTCCCTA212
27.3 GACCGAC103
28.3 CATGCCA184
28.7 CTCCGAC339
29.9 CATGTTC207
30.6 CATGATC335
30.7 CTCCGAC99
32.8 CTCCGAC216
33.9 CCATCGC171
34.3 CCATCAT146
35.0 CATGTTC205
35.3 CCATCTG198
35.7 GACCAAG93
37.4 CATGGAT231
39.6 CTCCGAC329
40.5 CCATCGC450
40.7 CATGTTC195
42.5 GACCAAG269
45.9 CCATCTG237
46.1 CATGGAT234
49.1 CCATCGC286
55.8 CATGATC265
75.1 CCATCTG191
GACCGAC255 0.0
CTCCCTA212 6.1
CATGCCA184 8.4
CTCCCTA342 10.0
CATGATC335 10.8
GACCGAC128
CTCCGAC216 12.9
CTCCGAC206 13.6
CATGGAT231 14.1
CATGTTC205 14.7
CCATCAT146
CCATCGC171
GACCAAG93 16.3
CTCCGAC329 17.4
CCATCCA354 18.4
CTCCAAG353 20.7
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CATGGAT234 27.0
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CTCCCTA117 40.9

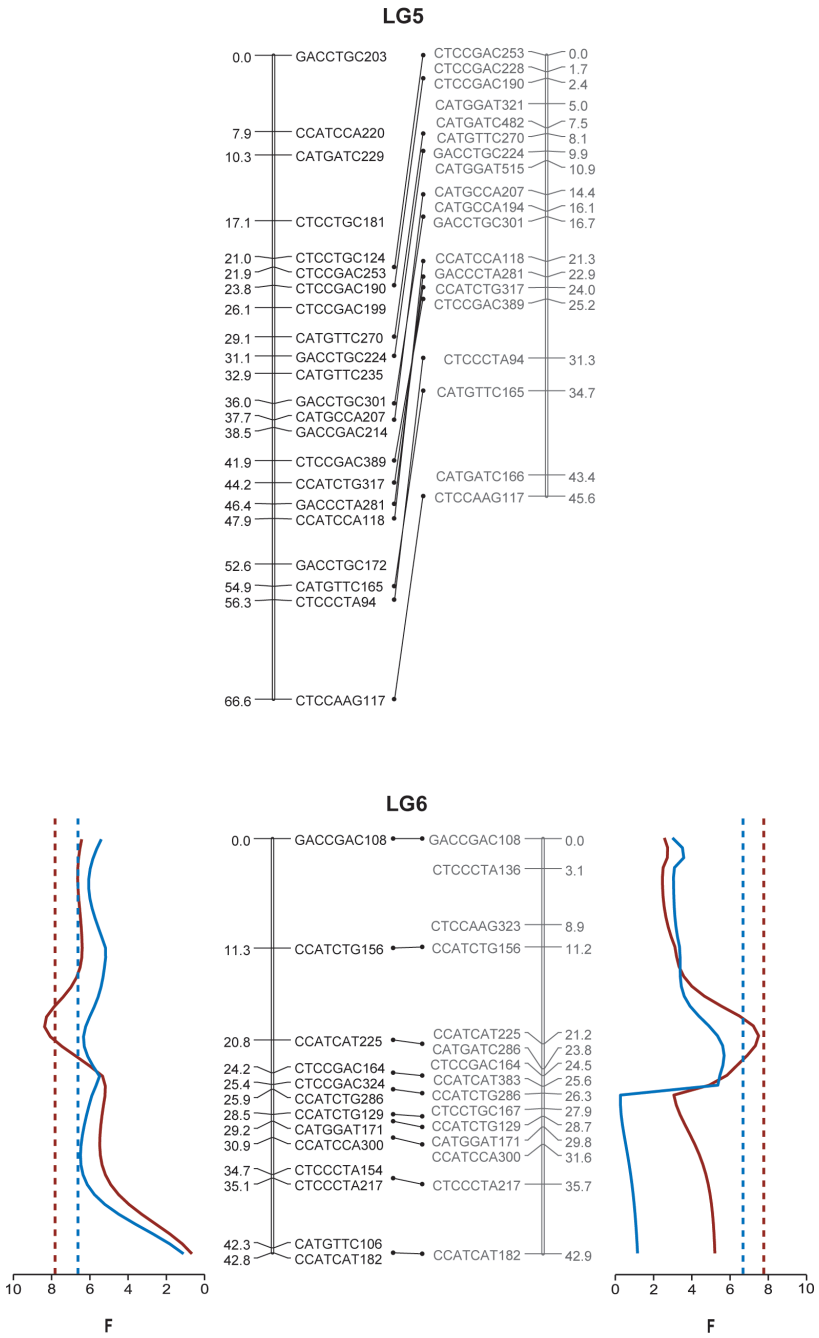

Figure 4: Estimated linkage map showing the positions of the detected QTL. Marker names and the corresponding relative genetic distances (cM) are shown alongside the vertical lines representing linkage groups (LG). Pairs of linkage groups from the maternal linkage map (black) and the paternal linkage map (grey) are connected by markers segregating in both parents (note that LG2 from the maternal map does not have a corresponding linkage group in the paternal map). The profiles of the *F*-statistic are shown only for those LG showing suggestive or significant QTL (blue = body size; red = beak length). Dotted lines represent the 1% chromosome-wide significance thresholds as determined by permutation tests (see text).

Accordingly, the Grouping function of JoinMap 4.0 split the maternal markers into six linkage groups and the paternal markers into five linkage groups at LOD of 4, encompassing 341 cM and 232 cM, respectively (Figure 4). While the paternal map likely represents the five pairs of autosomes, the maternal map has an extra linkage group (LG2) that contains sex-determining QTL (see below), and we believe that it represents the X chromosome.

The recovered linkage groups in the maternal map ranged from 43 cM to 75 cM (mean: 56.8 cM) with an average density of 20.5 markers per group (range: 11 – 31). Similarly, the size of the linkage groups in the paternal map varied from 41 to 55 cM (mean: 45.3 cM) with a mean density of 17.2 markers per linkage group (range: 14 – 22). The mean distances between adjacent loci were similar in the maternal (mean ± se: 2.91 cM ± 0.27 cM) and paternal (2.79 cM ± 0.31 cM) maps, and the longest distance between adjacent loci was 19.34 cM on the maternal LG 4. Thus, although in many species the frequency of recombination differs between sexes, we found no evidence that this is the case in *J. haematoloma.*

The total map length of *J. haematoloma* seems to be short for an insect with an estimated genome size of 1.79 Gb (Andrés, unpublished). Several molecular and cytogenetic characteristics are likely to contribute to the observed reduced recombination rates: First, *J. haematoloma* autosomal bivalent chromosomes usually show only one terminal chiasma (Bressa *et al.* 2001). Second, chromosomes are holocentric, lacking centromeres, and this structure may be prone to reduced recombination (Bressa *et al.* 2001, 2005). Third, at least in the male germ line univalent autosomes can be relatively common (Bressa *et al.* 2001). Our short linkage maps are consistent with these characteristics.

The behavior of the largest pair of autosomal chromosomes in *J. haematoloma* is noteworthy. This pair of chromosomes can be often observed as univalents or as a bivalent with terminal chiasmata, resulting in a large area of reduced recombination around the center of the chromosome (Bressa *et al.* 2001). Thus, one might *a priori* expect a map with at least one linkage group showing spatially aggregated markers around the center. The observed patterns are

consistent with this prediction. While in five (out of six) linkage groups the positions of the AFLP markers do not deviate significantly from a random distribution (χ^2 goodness-of-fit, $P > 0.05$), LG 4 shows a significant aggregation of markers ($P < 0.001$) around the center in both the paternal and the maternal maps.

2.3.2 Genetic architecture of beak and body lengths

When the genetic architecture of a trait is simple, rapid adaptation can occur through the fixation of a few mutations of large effect (Orr & Coyne 1992; Orr 2005; Barrett & Schluter 2008). Thus, traits involved in contemporary evolution may be controlled by only a few genes with major effects on the phenotype. Such an oligogenic model predicts that beak length should be controlled by only a few QTL. Our interval mapping analysis using a one-QTL model revealed only one significant QTL for beak length on LG 6 (maternally at $P_{\text{EXPERIMENT-WIDE}} < 0.01$ and paternally at $P_{\text{CHROMOSOME-WIDE}} < 0.05$). In both maps, the markers with the highest F-value associated with this QTL occupied the same position (maternal position: 19 cM; paternal position: 20 cM; Figure 4), and in both cases the detected QTL had a moderate effect (about 15%) on beak length ($V_{\text{EXPLAINED-MATERNAL}} = 15.7\%$; $V_{\text{EXPLAINED-PATERNAL}} = 14.1\%$). Using this QTL as background genetic effects, no more QTL could be found in any of the other linkage groups. Similarly, a two-QTL model also failed to find any other loci associated with beak length on LG6. Including QTL × sex interactions in the one-QTL model had no significant effect on the residual phenotypic variance, suggesting that the effects of this QTL are similar in both sexes. Our single marker regression analyses found nine markers associated with beak length. After controlling for sex differences, the percentages of beak length variance explained by these markers ranged from 5-14% (average 7%, Table 1). Our results, therefore, are consistent with an oligogenic model in which the rapid evolution of beak length to better match the fruit size of a newly introduced host is controlled by a limited number of loci of substantial effect. However, the number and effect of QTL observed in our study have to be interpreted cautiously. First, the

relatively small size of our mapping family results in a limited power to detect QTL of small effects and an overestimate of the effect of the detected ones (Beavis 1998; Xu 2003). This is also true for QTL found in regions showing low recombination rates (Noor *et al.* 2001). Second, the number, position and effect of QTL may be specific for the parental populations analyzed and further QTL may be found in different genetic backgrounds. These potential caveats do not necessarily compromise our results. However, further studies are needed to get a definitive picture of the number of genetic elements determining beak length in soapberry bugs.

From an adaptive perspective, differences in beak length are the most interesting because of the clear ecological relevance of this trait. Yet, bugs colonizing the introduced tree differ from the ancestral bugs in a variety of morphological and physiological traits. Previous studies have shown that although there are no significant differences in body size between bugs feeding on the introduced and native host (Carroll & Boyd 1992), hybrid lines with relatively longer beaks tend to be bigger (Carroll *et al.* 2001), suggesting that these two traits are not completely independent. The beak length QTL found on LG 6 co-localizes with a suggestive QTL for body size ($P_{\text{CHROMOSOME-WIDE}} = 0.02$, $V_{\text{EXPLAINED}} = 12.2\%$; Figure 4), indicating either linkage disequilibrium between two different beak and body size QTL or a single QTL with pleiotropic effects. In this case, including QTL × sex interactions in the one-QTL model had significant effect on the residual body length variance, indicating that the effects of this QTL are different between sexes. Controlling for the effect of this QTL on LG 6, we found two more QTL related to overall body size differences in the maternal map (Figure 4). The first of these QTL is located on the putative X chromosome (LG 2) and had a moderate effect on body length ($P_{\text{EXPERIMENT-WIDE}} < 0.01$, $V_{\text{EXPLAINED}} = 11.8\%$). The second one is located in LG1 ($P_{\text{CHROMOSOME-WIDE}} < 0.01$, $V_{\text{EXPLAINED}} = 5.9\%$). Single marker regression analyses found another three markers significantly associated with body length. After controlling for sex, the percentages of body size variance explained by these markers ranged from 8-13% (average 9%). Interestingly, two of them (CCATCAT186 and CCATCTG199, Table 1) had significant effects on both beak length and body size. Even more

interestingly, CCATCTG199 showed opposite effects on the two traits. This again shows that though developmentally and/or genetically interrelated, these two traits have different genetic architectures.

Table 1: Results of single marker regression analysis using ANCOVA on distorted and ungrouped markers. Markers in bolds are those that were not grouped into any existing linkage group; the other markers were excluded from the map because they showed significant segregation distortion ($P < 0.05$). Effect: +, increases beak/body length; −, decreases beak/body length. %: percentage of the trait variation explained by this marker. *P*: p-value. * denotes that a significant interaction between sex and marker was present. ns: non-significant.

Marker	**Beak length**			**Body size**		
	Effect	***P***	**%**	**Effect**	***P***	**%**
CCATCAT186	−	0.021	6.7	+	0.009*	8.4
CCATCTG199	−	0.036	5.5	−	0.001	13.0
CCATCTG241	−	0.020	6.4	ns	ns	ns
CTCCCTA146	−	0.005	9.7	ns	ns	ns
CTCCCTA204	−	0.022	6.6	ns	ns	ns
CTCCAAG178	−	0.048	4.9	ns	ns	ns
CCATCGC293	−	0.017	7.1	ns	ns	ns
GACCGAC234	+	0.008*	8.7	ns	ns	ns
CATGTTC148	ns	ns	ns	−	0.035	5.6

Our findings altogether revealed a complex genetic architecture underlying beak diversification in soapberry bugs. Former studies showed that differences in beak length involved a substantial amount of both additive and non-additive, particularly epistatic, genetic variation (Carroll *et al.* 2001; Carroll 2007). Thus a *priori*, we expected to detect significant QTL

× QTL interactions. In contrast, with the two-dimensional two-QTL genome scan using the Haley-Knott regression method in R/qtl, we could not find any potential QTL interactions for beak length. This apparent contradiction between our results and those of previous studies is likely to be the result of our low power to detect epistasis. Detecting epistasis is far more difficult than detecting single QTL and requires relatively big samples sizes (n > 400), especially in the case of interactions involving dominance effects (Mao & Da 2005; Wei *et al.* 2010). Dominance is an important component of variance in beak length in soapberry bugs (Carroll *et al.* 2001; Carroll 2007). Therefore, it is not entirely surprising that we could not detect any significant epistatic effects. Similarly, our two-dimensional two-QTL genome scan for body size could not detect any significant QTL interactions.

2.4 Conclusion

Contrasting views still exist on the number of underlying loci and magnitude of allelic effects involved in adaptation in natural populations. At the two ends of a continuum, adaptive evolution can be driven by changes in many genes of minor effect (polygenic model), or by mutations in a few genes of major effect (oligogenic model; Orr & Coyne 1992; Orr 2005). While rapid large phenotypic shifts observed in *Jadera* beaks suggest the existence of loci of relatively large effects (Orr 2005; Barrett & Schluter 2008), comparative and experimental evidence (Carroll, Dingle, *et al.* 2003; Stern & Orgogozo 2009) also points towards the presence of small effects and epistatic loci. A major contribution of our work is the assessment of the number of loci involved in beak reduction. Our results support an oligogenic control of beak length. Our findings are relevant for understanding rapid evolution of beak length differences associated with other anthropogenic host-shifts, such as the Australian Red-eyed bug, *Leptocoris tagalicus*, which has colonized two introduced species of invasive balloon vines that have much larger fruits than the native hosts (Carroll 2007).

CHAPTER 3 - CONCLUSION AND FUTURE DIRECTIONS

The soapberry bug ecomorphs diverged over very short evolutionary time after a biotic invasion. By constructing the first genome-wide linkage map available for *Jadera haematoloma*, the present study helped elucidate the genetic architecture underlying the rapid ecological divergence by identifying genomic regions potentially associated with the QTL controlling the beak length, an ecologically important trait that directly affects feeding in the soapberry bug.

The limitations on the accuracy of the linkage maps were largely imposed by the use of dominant AFLP markers and non-inbred parents. As a result of using dominant markers, the frequencies of one of the homozygous genotype and the heterozygous genotype had to be estimated. To complicate matters, non-inbred parental lines meant that the linkage phases also had to be estimated. To obtain linkage maps with finer details and higher accuracy, future studies should use codominant markers, most preferably SNP. The advent of next generation sequencing technologies has opened new possibilities in the analysis of complex traits. These new technologies have exponentially increased the quantity of sequences generated, producing up to several million bases in a single run and making it feasible to generate high-density maps at a reasonable cost. The ability to quickly generate high-density maps is particularly relevant for the study of complex traits in non-model organisms, such as the soapberry bug, where inbred lines are not likely to be available. Mapping outbreeding lines is possible using SNP next generation sequencing data as only a fraction of the SNP markers would have unknown linkage phases, which would be negligible considering that the map would be most likely saturated with other markers. Saturated maps give much higher power to fine-map detected QTL (see Evans & Cardon 2004).

Alleles at any given locus may vary between non-inbred individuals. Depending on which individuals are used for crossing, the resulting F2 populations may have different sets of segregating QTL. Therefore, when looking at the genetic architectures of complex traits of

non-model organisms using outbred populations, it is also desirable to establish several mapping families. Different sets of segregating QTL from different families can potentially be detected and provide a more comprehensive view of the QTL involved in ecologically important traits. Next generation sequencing technologies are able to sequence mixes of individually tagged DNA, allowing for easy genotyping of hundreds of individuals that are required for having enough power to detect epistatic interactions (Mao & Da 2005; Wei *et al.* 2010). Also, many QTL analysis programs are able to consider multiple families simultaneously.

The field is moving toward predictive studies, using models to predict the process and outcome of evolution. Current models range from a few locations of genomic divergence to wide-spread genomic divergence. To be able to characterize the genomic architecture of adaptive divergence in the soapberry bug, the genome-wide distribution pattern of highly differentiated markers between the ecomorphs could be studied. It is expected that many of the highly differentiated markers are clustered around the identified QTL for beak length and body length, undetected QTL for the same traits, and QTL underlying other differentiated traits.

Comparative studies are also important in increasing the predictive power of proposed models. While there was a shortening of the beak in *Jadera haematoloma* as a consequence of exploiting a new host, similar cases of another soapberry bug species in Australia showed beak elongation (Carroll *et al.* 2005). Adaptation to new host plants induced evolution in opposite directions. Characterizing and comparing the genetic architectures in the two cases would provide insights into the genetic basis to adaptive divergence.

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